

Investigation of the effects of stobadine on the antioxidant enzymes in carbon tetrachloride mediated brain toxicity

[Karbon tetraklorür aracılı beyin toksisitesinde antioksidan enzimler üzerine stobadinin etkisinin araştırılması]

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ABSTRACT

Objectives: The aim of the present study was to investigate the relation between brain damage induced by carbon tetrachloride and superoxide dismutase, catalase, glutathione peroxidase, and glutathione S-transferase enzyme activities. Also, the other aim was to evaluate the effect of stobadine on this metabolism. We particularly choose brain tissue as an experimental model because it contains reach polyunsaturated fatty acids which is one of the most important targets of the free radicals. Another important reason for choosing brain as a model is the limited studies with brain tissue damaged with carbon tetrachloride that compared to numerous ones with hepatic tissue.

Methods: Rats were randomly separated into four experimental groups as control (n=10), stobadine (n=10), carbon tetrachloride (n=10), carbon tetrachloride+ stobadine (n=10).

Results: Carbon tetrachloride administration reduced superoxide dismutase, catalase, glutathione peroxidase, and glutathione S-transferase enzyme activities. Catalase, superoxide dismutase and glutathione S-transferase enzyme activities of the carbon tetrachloride+ stobadine group nearly approached the levels of the control group. On the contrary, glutathione peroxidase activity could not access the normal levels. Histopathologic results showed that carbon tetrachloride induced morphologic damage and edema in the brain cells. In spite of limited improvement in the carbon tetrachloride + stobadine group we found eosinophilia and edemas at the neurons and neuroglial cells, respectively.

Conclusions: It could be suggested that glutathione peroxidase enzyme can be more important antioxidant enzyme to protect brain cells against the oxygen or carbon tetrachloride based free radicals.

The authors do not have a conflict of interest.

Key Words: Carbon tetrachloride, antioxidant enzymes, brain and stobadine

ÖZET

Amaç: Bu çalışmada, karbon tetraklorür kaynaklı beyin hasarı ve süperoksid dismutaz, katalaz, glutatyon peroksidaz, glutatyon S-transferaz enzim aktiviteleri arasındaki ilişkiyi ve stobadinin bu metabolizma üzerindeki etkisini araştırmak amaçlandı. Beyin dokusu, serbest radikaller için çok önemli bir hedef olan poliansature yağ asitlerinden zengin olduğu için deneysel model olarak tercih edildi. Ayrıca beyin dokusunda karbon tetraklorür hasarıyla ilgili sınırlı sayıda çalışmanın olması da bir model olarak beyin dokusunun seçilmesi açısından önemli bir nedendir.

Gereç ve yöntemler: Ratlar; kontrol (n=10), stobadin (n=10), karbon tetraklorür, karbon tetraklorür+stobadin (n=10) gibi dört grup şeklinde rastgele gruplandı.

Bulgular: Karbon tetraklorür uygulanan grupta süperoksid dismutaz, katalaz, glutatyon peroksidaz, glutatyon S-transferaz enzim aktivitelerinin azaldığı görüldü. Karbon tetraklorür +stobadin grubunda katalaz, süperoksid dismutaz ve glutatyon S-transferaz enzim aktivitelerinin kontrol grubu düzeylerine yaklaştığı izlendi. Öte yandan glutatyon peroksidaz aktivitesi ise normal düzeylere ulaşamadı. Histopatolojik sonuçlar karbon tetraklorür uygulamasının beyin hücrelerinde morfolojik hasara ve ödeme neden olduğunu gösterdi. Karbon tetraklorür +stobadin grubunda sınırlı bir iyileşme olmasına rağmen nöronlarda eozinofili ve nöroglial hücrelerde ödem devam ediyordu.

Sonuç: Bu bulgulara göre glutatyon peroksidaz enziminin karbon tetraklorür kaynaklı radikallere karşı beyin hücrelerini korumada diğer antioksidan enzimlerden daha önemli olduğu sonucuna varılmıştır.

Yazarların çıkar çatışması bulunmamaktadır.

Ahtar Sözcükler: Karbon tetraklorür, antioksidan enzimler, beyin ve stobadin

Introduction

Oxidative stress is known to cause major derangements of cellular metabolism, including modifications of protein and nucleic acid structure, damage to membrane ion transport and permeability, destruction of the cells by lipid peroxidation [1,2]. Balance between free radical activity and anti-oxidant defense system becomes an important requirement for preventing damage to the cellular membrane caused by oxidative stress. These antioxidant systems include nonenzymatic antioxidants like glutathione, uric acid, bilirubin, vitamin C and E and enzymatic activities such as that of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSHPx) [1,3,4].

Glutathione (GSH) and enzymes cooperating with it, GSHPx, glutathione S-transferase (GST) and glutathione reductase (GSHR), play crucial role in cell defense against reactive oxygen species. GSHPx plays an important role in the defense against free radicals, peroxides, a wide range of xenobiotics and carcinogens [5]. GST is a family of substrate-specific enzymes that catalyze GSH conjugation; thereby they potentially reduce radical toxicity [6]. Reduced glutathione is a notable reductant in the cell for it protects against free radicals, peroxides, and other toxic components. It helps maintaining the normal structure and function of cells, probably by its redox and detoxification reactions [7]. It has been suggested that liver necrosis is initiated when GSH reserves are markedly depleted [8].

The limited capacity of antioxidant defense mechanisms in the brain as compared to peripheral tissues was reviewed by Del Maestro et al. [9]. The brain is rich in SOD but contains small amounts of CAT [10]. CAT is likely to be important only at sites of relatively high hydrogen peroxide (H_2O_2) concentrations, such as peroxisomes. Another antioxidant enzyme, selenium-containing enzyme GSHPx also converts H_2O_2 to water, oxidizing GSH to GSSG in the process. GSHPx reduces most of the H_2O_2 in the cytoplasm and it also converts organic peroxides to organic alcohols and water. GSHPx can also terminate the chain reaction of lipid peroxidation by removing lipid hydroperoxides from the cell membrane [5]. It is notable that central nervous system contains substantially more GSHPx than CAT [11].

Carbon tetrachloride (CCl_4) which is a lipid-soluble potent hepatotoxic agent took part in human medicine as an anesthetic agent and is extensively used as an industrial solvent [12]. The liver injury caused by CCl_4 may be due to the free radical reaction with subsequent initiation of peroxidative degeneration of lipids and proteins in many tissues [3, 13, 14]. It is suggested that the toxicity of CCl_4 probably depends on formation of the trichloromethyl radical (CCl_3). In the presence of oxygen, CCl_3 reacts with it to form the more toxic trichloromethyl peroxide radical (CCl_3O_2) [2, 4]. Lipid peroxidation is initiated by the interaction of these reactive radicals of CCl_4

with unsaturated fatty acids of membrane lipids. Experimental studies showed that central nervous system is sensitive to CCl_4 like the hepatic tissue [15].

New antioxidants which have different mechanisms have developed to prevent the formation of reactive oxygen species or to reduce harmful effects of them and stobadine (ST, (–)-*cis*-2,8-dimethyl-2,3,4,4a,5,9b-hexahydro-1H-pyrido[4,3-b]indole) is one of these antioxidants [16]. It was shown to be able to scavenge hydroxyl, alkoxyl, and peroxy radicals and prevent superoxide radical generation. The antioxidant properties of stobadine are conditioned mainly by its indole ring compounds' ability of stabilizing radicals. Thus, oxygen reduces oxidative stress induced lipid peroxidation and protein oxidation [17, 18]. In addition, ST may repair oxidized amino acids, and preserve oxidation of SH groups by one-electron donation [19]. It was also shown that ST prevents lipid peroxidation in liposomes [20] in cerebral mitochondria, synaptosomes and microsomes [21] as well as in heart mitochondria after oxidative stress induced by isoprenaline [22]. ST maintained high GSHPx activity, probably by preventing glutathione oxidation [23].

In the present study, we examined the relation between CCl_4 toxicity and protective role of antioxidant enzymes in rat brain and also the effect of the ST on this metabolism. There is no modeling similar to our study's 'animal model' in the literature. Other reasons in the selection of our study model are; excess accumulation of CCl_4 in lipid-rich brain tissue due to its molecular properties, insufficient antioxidant defense mechanisms of brain compared to other organs and according to toxicity formed by radical molecules, which is more likely to affect the brain. Another important reason for choosing brain as a model is the limited studies with brain tissue damaged with CCl_4 that compared to numerous ones with hepatic tissue.

Material and Methods

Experimental animal and treatment protocol

This study was approved by Experimental Research Center, Gazi University, Ankara, Turkey. In the present study, we used 40 Wistar albino (250-300 gr) rats which were housed in wire bottom cages, free diet and with a 12 h light/dark cycle. The animals were divided into four groups. Each experimental group consisted of ten animals. Group 1 (n=10): Control group, they were fed with only standard rat diet for 8 week. Group 2 (n=10): They were treated with 24.7 mg/kg/day ST [dissolved in 0.5% Avicel (carboxymethyl cellulose) solution] three times per week for 8 weeks. Eight week by oral way (24, 25). Group 3 (n=10): They were treated three times a week (Monday, Wednesday, Friday) intraperitoneal CCl_4 [dissolved in olive oil 1/10 (v/v)] treatment, first week 0.3 ml/kg CCl_4 , second week 0.7 ml/kg CCl_4 , and 3 – 8 weeks 1 ml/kg CCl_4 (26). Group 4 (n= 10): They were treated with 24.7 mg/kg/day ST [dissolved in 0.5% Avicel

(carboxymethyl cellulose) solution] three times per week for 8 weeks. Eight week by oral way. They were treated three times a week (Monday, Wednesday, Friday) intraperitoneal CCl₄ [dissolved in olive oil 1/10 (v/v)] treatment; first week 0.3 ml/kg CCl₄, second week 0.7 ml/kg CCl₄, and 3 – 8 weeks 1 ml/kg CCl₄ [24-26]. After 24 hours from the last administrations, all rats were sacrificed under general anesthesia and muscle relaxant (40 mg/kg Alfamin and 2.5 mg/kg Alfazyme i.m.). Brains were harvested and cleaned with 0.9% NaCl solution. Brain tissue samples were stored in 10% formalin solution for histopathology analysis. The remaining brain tissues were immediately frozen in liquid nitrogen and stored in a deep freezer at -80 °C until all measurements.

Study design

Firstly, 1000 mg of brain tissue samples were homogenized with 4 cc 0.9% NaCl at 4000 rpm and then the extracts were centrifuged at 16000 x g +4 °C for 20 minute. Parts of the supernatants were separated for the determination of protein and catalase enzyme activity. Remaining supernatants were mixed with 5 / 3 (v / v) ethanol / chloroform mixture with 1 / 1 ratio and again centrifuged at 16000 xg (12000 RPM) at +4°C for 20 minutes using a refrigerated centrifuge. Top phase was left for SOD, GSH-Px and protein determination.

Analysis of brain enzyme activities

SOD activity was measured by the method of Durak *et al.* [27]. GSHPx activity was measured by method of Paglia and Valentine [28]. CAT activity was measured by Aebi [29]. GST activity was assayed by Habig *et al.* [30]. Enzyme activities were reported as U/mg protein for SOD, IU/mg protein for CAT and GSHPx and mIU/mg protein for GST. Protein concentrations of the tissue homogenates were measured by Lowry method [31].

Brain preparation for histopathology

The specimens were fixed in 10% formalin solution for 72 hours. After fixation, the tissues were washed under running tap water for 24 h and dehydrated with 50, 60, 70, 80, 90, 96 and 100% concentrated ethanol, respectively. The specimens were then laid in a 1:1 ratio of immersion oil and absolute alcohol for 1h, followed by immersion oil overnight for transparency. After the application of xylol, the specimens were made into paraffin blocks using a 1:1 xylol and paraffin mixture for 1h and paraffin for 6h in an incubator. 10 micron thick sections were rehydrated and dyed with Masson's trichrome (Bio-Optica Masson tricromica cat no 04-010802, Milano S.p.a, via San Faustino, 58,20134 Milano, ITALIA) technique.

Statistical Analysis

Data were presented as the mean ± standard deviation. Data were analyzed by Kruskal-Wallis and Mann-Whitney U test.

Results

SOD, CAT, GSHPx, and GST enzyme activities were suppressed by CCl₄ administration. SOD, CAT and GST activities in the CCl₄+ST group nearly approached the levels of control group. GSHPx activity level was low in the CCl₄+ST group compared with the control group. SOD, CAT, GSHPx and GST activities were significantly increased in the CCl₄+ST group compared with CCl₄ group (Table 1). Histopathology results showed that CCl₄ administration caused degeneration of neuronal morphology, cytoplasmic eosinophilia, detachment of neuroglial tissue and edema in the brain cells (Figure 1). Tissue morphology was normal and similar in control and ST groups (Figure 2, 3). Eosinophilia and edemas at neurons and neuroglial cells were persisted in the CCl₄+ST group (Figure 4).

Discussion

After CCl₄ administration, it is distributed and deposited to organs such as the liver, brain, kidney, and heart. CCl₄ is rapidly taken up by the liver and brain [32]. The major injury after CCl₄ intoxication was investigated in the liver. So there are many experimental studies about liver toxicity of CCl₄, but still there are only limited studies with the brain tissue.

Vohra and Hui [33] studied CCl₄ toxicity in cultured neurons and showed lactate dehydrogenase (LDH) release and TBARS content increase. Conversely, they found that GSHPx activity decreased when CCl₄ was added to the neuron culture media. The increase in thiobarbituric acid reactive substances (TBARS) in cultured neurons after CCl₄ treatment is in accordance with the studies made by Clemedson *et al.* [34]. The increased level of TBARS is an indicator of the oxidative damage caused by CCl₄. Besides they reported an increase in lipid peroxidation in the CCl₄ treated neuronal cultures. Addition of CCl₄ to cultured neurons resulted in wide spread neuronal death. Jayakumar *et al.* [35] investigated the putative antioxidant activity of oyster mushroom *Pleurotus ostreatus* and observed that administration of extract of *P. ostreatus* was improved the heart, kidney and brain damage caused by CCl₄ mediated acute oxidative stress. They showed that *P. ostreatus* reduced lipid peroxidation and improved enzymatic/non-enzymatic antioxidant defense mechanism activities. Histopathological studies confirmed the antioxidant effect conferred by the extract of *P. ostreatus*. Pentyala *et al.* [36] demonstrated that CCl₄ effected signal transduction pathways. They suggested that it may exert neurotoxicity by altering calcium mechanism and protein kinase C. The study of Pentyala *et al.* might not be directly similar with our study. However, it supports us in terms of the explanation of neurotoxicity caused by CCl₄ with a different mechanism. The cause of toxicity is the accumulation of lipid-soluble CCl₄ in lipid-rich brain tissue as Sangrizi *et al.* [32] indicated in their study. CCl₄ neurotoxicity may

Table 1. Effect of stobadine on SOD, CAT, GSHPx, GST activities of rat brain tissues treated with CCl₄

GROUPS	SOD (U/mg. prot.)	CAT (IU/mg. prot.)	GSH-Px (IU/mg. prot.)	GST (mIU/mg.prot.)
Control	44.90±24.98	8.19±2.38	0.51±0,19	3.32±1.31
ST	36.39±20.20	5.41±2.13	0.40±0,15	3.31±0.51
CCl₄	11.54±5,47 ^a	3.97±2.48 ^a	0.10±0.053 ^a	1.72±0.49 ^a
CCl₄ +ST	28.24±13.54 ^b	10.19±3,80 ^b	0.27±0.10 ^{b,c}	3.69±1.95 ^b

^a p<0.01 CCl₄ group compared with control group

^b p<0.01 CCl₄+ST group compared with CCl₄ group

^c p<0.05 CCl₄+ST group compared with control group

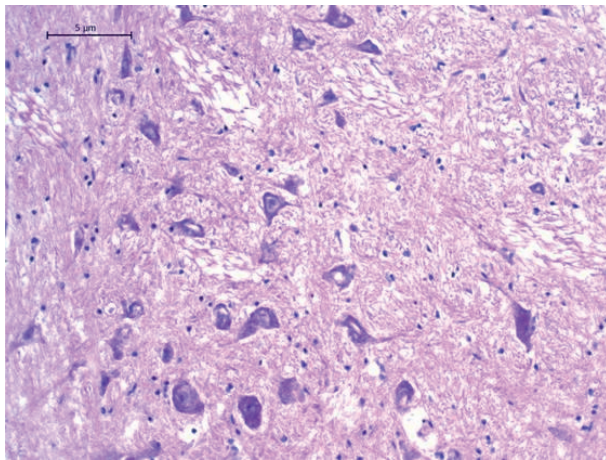


Figure 1: Brain CCl₄. Degeneration of neuronal morphology, eosinophilic cytoplasm, detachment of neuroglial tissue and edema is illustrated (Bar: 5µm, Hematoxylin & Eosin)

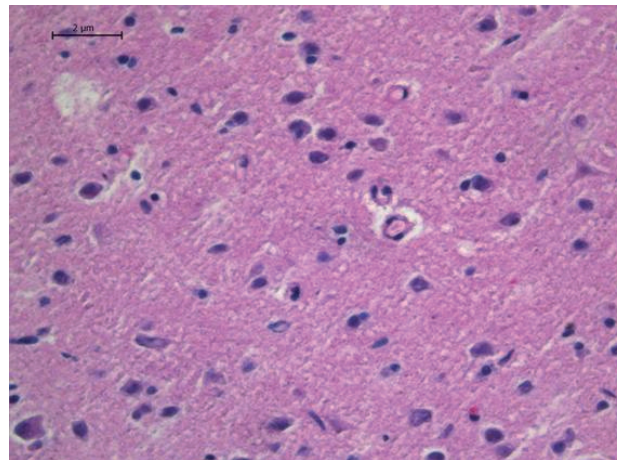


Figure 2: Brain Control. Normal nervous tissue. (Bar: 2µm, Hematoxylin & Eosin)

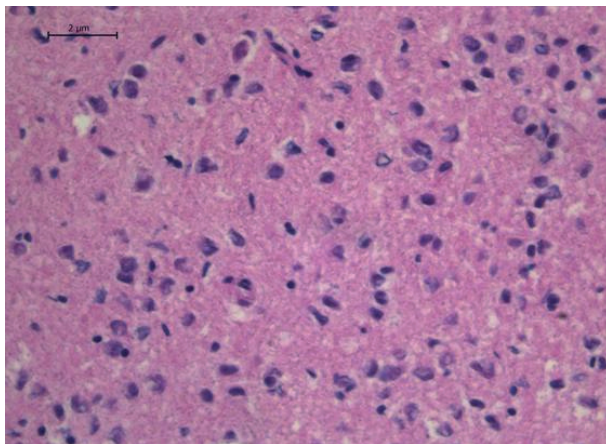


Figure 3: Brain Stobadine. Morphology of this group was similar to that of normal control. (Bar: 2µm, Hematoxylin & Eosin)

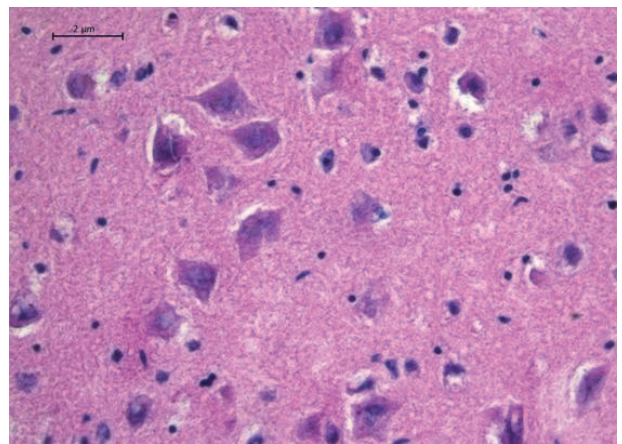


Figure 4: Brain CCl₄+Stobadine. Eosinophilic cytoplasm of neurons, edema, integrity of neuroglial tissue is demonstrated (Bar: 2µm, Hematoxylin & Eosin)

be due to its molecular weight, membrane permeability and lipid solubility properties. The brain may have different coping mechanisms to deal with oxidative stress than liver. Ahmad et al. [37] demonstrated free radical formation by using spin trapping techniques in the brain after CCl_4 administration. Although the amount of free radicals formed is considerably lower as compared with those generated in the liver and kidney, the effect of free radicals on the brain membranes may be serious due to its sensitivity to free radical attack or lipid peroxidation which is resulting in brain dysfunction and cerebral edema [38, 39].

It has been reported that the enzymatic antioxidant defense system has limited capacity in the brain tissue [9, 40]. It is known that the brain tissue contains small amounts of CAT and GSHPx enzyme activities which can also interact directly with lipid peroxides. Moreover, the central nervous system contains substantially more GSHPx than CAT [40]. Adams et al. [40] suggested that GSHPx is more important than CAT for detoxification of H_2O_2 in the brain. The high concentration of polyunsaturated fatty acids and aerobic metabolic activity of the brain increase the susceptibility of this organ. For this reason, the brain tissue easily could be a target to peroxidative damage induced by reactive oxygen species after CCl_4 ingestion [9, 40].

In our study, in order to protect the brain tissue from CCl_4 toxicity, ST was selected as an antioxidant molecule because of its indole ring structure which carries antioxidant properties. ST was shown to be able to scavenge hydroxyl, peroxy, and alkoxy radicals in the literature [17, 41]. ST was preferred in this study because of its putative antioxidant effects on CCl_4 induced formation of carbon-based radicals and consequently nitrogen, peroxy or alkoxy radicals. Any literature study has been observed which used stobadine for its antioxidant effect in order to prevent free radical damage caused by CCl_4 . In this respect, our study has the distinction of being the first study investigating the effects of ST on radical damage formed by CCl_4 . Our study is also remarkable in terms of showing CCl_4 induced radical damage to the brain tissue both enzymatically and histopathologically.

All enzyme activities in CCl_4 group showed significant suppression than the control group in our study. When $\text{CCl}_4 + \text{ST}$ group was compared with the control group; although SOD, CAT and GST activities in the $\text{CCl}_4 + \text{ST}$ group nearly approached the levels of control group, radical damage was not completely improved in the $\text{CCl}_4 + \text{ST}$ group. Additionally, only GSHPx activities were suppressed among the enzyme activities. These results suggested that GSHPx may be enzymatically important to the brain tissue. As Histopathological improvement was not seen in $\text{CCl}_4 + \text{ST}$ group, GSHPx suppression becomes more significant. The other results including low CAT activity in the brain tissue, peroxy radical which is used as a substrate with H_2O_2 by CAT whereas the only substrate of CAT is H_2O_2 , are also important. CCl_3 and

CCl_3O_2 radicals are responsible for the toxic effects of CCl_4 and they react with oxygen-based radicals to form organic peroxide radicals [2,4]. These radical structures easily lead to superoxide anion and superoxide anion reacts with SOD to form H_2O_2 . Haber-Weiss and Fenton reactions generate hydroxyl radicals from H_2O_2 even if H_2O_2 has no radicalic properties. GSHPx detoxifies H_2O_2 and organic peroxides by using them as substrates [42,43]. That's why the catalytic activities of GSHPx make it a key enzyme for radical metabolism.

GSHPx may be important for scavenging peroxides produced by CCl_4 -based radicals. Our results seem to support these data. GSH is likely to be the limiting substrate for regulation of GSHPx activity. GST is one of the two enzymes which use reduced glutathione with GSHPx. Because of the higher reaction rate of GST than GSHPx, GST rapidly depletes GSH [42]. GSHPx may show failure to detoxify peroxy radical in the same status.

In our study there are remarkable results such as; GSHPx activity was suppressed in the $\text{CCl}_4 + \text{ST}$ group compared with control group and ST failed to prevent cellular damage in $\text{CCl}_4 + \text{ST}$ group. These results' biochemical mechanism might be explained by the increased GST activity in $\text{CCl}_4 + \text{ST}$ group according to the decreased GSH levels and suppressed GSHPx activity. Because of this GSHPx becomes a regulator enzyme which prevents CCl_4 induced brain toxicity with a different mechanism from CAT. Increased SOD levels in $\text{CCl}_4 + \text{ST}$ group contributed to increase H_2O_2 levels. CAT and GSHPx detoxification of H_2O_2 is the most important enzymatic defense mechanisms. CAT is an important enzyme in oxidative stress but CAT uses only H_2O_2 as a substrate with a limited capacity and has organelle limitations [44]. Ng et al. [45] indicated that CAT effects at low H_2O_2 levels whereas GSHPx effects at high H_2O_2 levels. Decreased GSHPx activity in $\text{CCl}_4 + \text{ST}$ group leads to increase at H_2O_2 and peroxy radical levels. These increases can make the brain vulnerable to radical toxicity. In the same group, H_2O_2 concentrations may suppress GSHPx activity and cause substrate inhibition. In this way, H_2O_2 may contribute to decrease at GSHPx enzyme activity and emergence of toxic effects. These two mechanisms may have regulator role in cellular GSHPx activation. Histopathologic results demonstrated that tissue damage by CCl_4 administration was scantily removed. However, eosinophilia at neurons and edemas at neuroglial cells were persisted in the $\text{CCl}_4 + \text{ST}$ group. Consequently, it was found that antioxidant ST could not improve the CCl_4 toxicity.

Our study is a valuable pilot study because it establishes antioxidant enzymatic mechanisms of brain toxicity, analyses putative protective properties of an antioxidant molecule stobadine and uses histopathologic evidences for monitoring tissue damage.

It is also important to investigate H_2O_2 and GSH levels which have key roles in radical metabolism and predicti-

ve damage markers like conjugated diens, modified bases (i.e hydroxy guanine) and oxidized protein products for elucidating the mechanisms.

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References

- [1] Recknagel RO, Glende EA Jr, Dolak JA, Waller RL. (1989) Mechanism of carbon tetrachloride toxicity. *Pharmacol Ther.* 43(1): 139–54.
- [2] Hartley DP, Kolaja KL, Reichard J, Petersen DR. (1999) 4-Hydroxynonenal and malondialdehyde hepatic protein adducts in rats treated with carbon tetrachloride: immunohistochemical detection and lobular localization. *Toxicol Appl Pharmacol.* 161(1):23–33.
- [3] Castillo T, Koop DR, Kamimura S, Triadafilopoulos G, Tsukamoto H. (1992) Role of cytochrome P-450 2E in ethanol-, carbon tetrachloride- and iron-dependent microsomal lipid peroxidation. *Hepatology.* 16(4): 992-6.
- [4] Melin AM, Perromat A, Deleris G. (2000) Pharmacologic application of Fourier transform IR spectroscopy: in vivo toxicity of carbon tetrachloride on rat liver. *Biopolymers.* 57(3):160–8.
- [5] Jung K, Henke W. (1996) Developmental changes of antioxidant enzymes activity in kidney and liver from rats. *Free Radic Biol Med.* 20(4): 613–7.
- [6] Hayes JD, Pulford DJ. (1995) The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Crit Rev Biochem Mol Biol.* 30(6): 445-600.
- [7] Guerri C, Grisolia S. (1980) Influence on prolonged ethanol intake on the level and turnover of alcohol and aldehyde dehydrogenase and glutathione. *Adv. Exp. Med. Biol.* 126: 365-84.
- [8] Recknagel RO, Glende EA, Britton RS. (1991) Free radical damage and lipid Peroxidation. *Hepatotoxicology (Meeks RG, ed.)*, p. 401–436. CRC Press, Boca Raton, FL.
- [9] Del Maestro R, Mc Donald W. (1987) Distribution of superoxide dismutase, glutathione peroxidase and catalase in developing rat brain. *Mech Ageing Dev.* 41(1-2): 29-38.
- [10] Hartz JW, Funakoshi S, Deutsch HF. (1973) The levels of superoxide dismutase and catalase in human tissues as determined immunochemically. *Clin Chim Acta.* 46(2): 125-32.
- [11] Watson BD, Busto R, Goldberg WJ, Santiso M, Yoshida S, Ginsberg MD. (1984) Lipid peroxidation in vivo induced by reversible global ischemia in rat brain. *J Neurochem.* 42(1): 268-74.
- [12] Jones IW. (1983) Chloroform anaesthesia in Liverpool. *Anaesthesia.* 38(6): 578-80.
- [13] Cabre M, Camps J, Paternain JL, Ferre N, Joven J. (2000) Time course of changes in hepatic lipid peroxidation and glutathione metabolism in rats with carbon tetrachloride-induced cirrhosis. *Clin Exp Pharmacol Physiol.* 27 (9): 694–99.
- [14] Simile MM, Banni S, Angioni E, Carta G, De Miglio MR, Muroli MR, Calvisi DF, Carru A, Pascale RM, Feo F. (2001) 5'-Methyl thioadenosine administration prevents lipid peroxidation and fibrogenesis induced in rat liver by carbon tetrachloride intoxication. *J Hepatol.* 34(3): 386–94.
- [15] Clemmedson C, Peterson A, Walum E. (1989) A combined in ovo-in vitro system for studies of volatile compounds on brain development: differential effects of carbon tetrachloride on neurons and astrocytes. *Pharmac. Toxicol.* 64(1): 94-9.
- [16] Stolic S, Bauer V, Benes L, Tichy M. (1983) Medicine with antiarrhythmic and antihypoxic activity and its method of preparation. Patents: CS 229 067, SWED. 8204693-9, BELG. 894148, SWISS 651 754, BRD P-3231088, SPAIN 553 017, JAP. 151 4040.
- [17] Stasko A, Ondrias K, Misik V, Szöchova H, Gergel D. (1990) Stobadine: a novel scavenger of free radicals, *Chem.* 44: 493-500.
- [18] Steenken S, Sundwust AR, Jovanovic SV, Crockett R, Sies H. (1992) Antioxidant activity of the pyridoindole stobadine: pulse radiolytic characterization of one electron oxidized stobadine and wuenching of singlet molecular oxygen, *Chem. Res. Toxicol.* 5:355-60.
- [19] Horakova L, Stolic S. (1998) Antioxidant and pharmacodynamic effects of pyridoindole stobadine. *Gen. Pharmacol.* 30(5): 627–38.
- [20] Ondrias K, Misik V, Gergel D, Stasko A. (1989) Lipid peroxidation of phosphatidylcholine liposomes depressed by the calcium channel blockers nifedipine and verapamil and by the antiarrhythmic-antihypoxic drug stobadine. *Biochim Biophys Acta.* 1003(3): 238- 45.
- [21] Horakova L, Sebokova B, Juranek I. (1989) International Conference on Regulation of Free Radical Reactions, Bulgaria, Varna, 13-16 Sept, Abstract Book, pp 75.
- [22] Ondrejickova O, Sedlac J, Macickova T, Benes L. (1988) Abstracts of the 14th International Congress of Biochemistry, vol II. Prague, July 10-15, p 130.
- [23] Horakova L, Lukovic L, Stolic S. (1990) Effect of stobadine and vitamin E on the ischemic reperfused brain tissue. *Pharmazie.* 45(3): 223-4.
- [24] Necas J, Bartosikova L, Benes L, Janostikova E, Bartosik T, Kluusakova J, Florian T, Frydrych M, Jurica J, Biomed. (2005) Pap. Med. Fac. Univ. Palacky Olomouc Czech Repub. 149(2):385-8.
- [25] Zimmerman HJ. (1978) The adverse effects of drugs and other chemicals in the liver hepatotoxicity, NewYork, Appleton Century Crofts.
- [26] Rozga J. (2001) Animal models of liver regeneration, In W.W. and D.G.Souba (Eds.), pp.703-707, Surgical Research, Wilmore California, Academic Press.
- [27] Durak I, Canbolat O, Kavutçu M, Oztürk HS, Yurtarslani Z. (1996) Activities of total, cytoplasmic, and mitochondrial superoxide dismutase enzymes in sera and pleural fluids from patients with lung cancer. *J Clin Lab Anal.* 10(1): 17-20.
- [28] Paglia DE, Valentine WN. (1967) Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med.* 70(1): 158-69.
- [29] Aebi H. (1974) Catalase. *Enzymatic Analysis*, (Berg Mayer HU (ed.)), p. 647-683, Weinheim Academic Press, New York.
- [30] Habig WH, Pabst MJ, Jakoby WB. (1974) Glutathione-S-transferases: The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* 249(22): 130-9.
- [31] Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951) Protein measurement with the folin phenol reagent. *J Biol Chem.* 193(1): 265–75.
- [32] Sanzgiri UY, Srivatsan V, Muralidhara S, Dallas CE, Bruckner JV. (1997) Uptake, distribution and elimination of carbon tetrachloride in rats tissues following inhalation and ingestion exposures. *Toxicol Appl Pharmacol.* 143(1): 120–9.
- [33] Vohra BP, Hui X. (2001) Taurine protects against carbon tetrachloride toxicity in the cultured neurons and in vivo. *Arch Physiol Biochem.* 109(1): 90-4.
- [34] Clemmedson C, Odland L, Walum E. (1990) Differential effect of carbon tetrachloride on the cell membranes of neurons and astrocytes. *Neurotoxicol Teratol.* 12(6): 597–602.

- [35] Jayakumar T, Sakthivel M, Thomas PA, Geraldine P. (2008) Pleurotus ostreatus, an oyster mushroom, decreases the oxidative stress induced by carbon tetrachloride in rat kidneys, heart and brain. *Chem Biol Interact.* 176(2-3):108-20.
- [36] Pentyala SN, Vig PJ, Sekhon BS, Desai D. (1994). Effect of carbon tetrachloride on inositol 1,4,5-trisphosphate dependent and independent regulation of rat brain microsomal Ca²⁺ flux. *Cell Signal.* 6(5):561-7.
- [37] Ahmad FF, Cowan DL, Sun AY. (1987) Detection of the free radical formation in various tissues after acute carbon tetrachloride administration in gerbil. *Life Sci.* 41(22): 2469–75.
- [38] Sun AY. (1972) The effect of lipoxidation on synaptosomal (Na⁺ + K⁺)-ATPase isolated from the cerebral cortex of squirrel monkey. *Biochim. Biophys. Acta.* 266(2): 350-60.
- [39] Watanabe A, Shiota T, Takei N, Fujiwara M, Nagashima H. (1986) Blood to brain transfer of carbon tetrachloride and lipo-peroxidation in rat brain. *Res. Commun. Chem. Path. Pharmacol.* 51(1): 137-40.
- [40] Adams JD Jr, Klaidman LK, Odunze IN, Shen HC, Miller CA. (1991) Alzheimer's and Parkinson's disease. Brain levels of glutathione, glutathione disulfide and vitamin E. *Mol Chem Neuro-pathol.* 14(3): 213–26.
- [41] Stolc S, Horakova L. (1988) *New Trends in Clinical Neuropharmacology.* (Bartko D et al (ed)), p. 59-63, J. Libbey and Co, London.
- [42] Meister A, Anderson ME. (1983) Glutathione. *Annu Rev Biochem.* 52:711-60.
- [43] Handy DE, Lubos E, Yang Y, Galbraith JD, Kelly N, Zhang YY, Leopold JA, Loscalzo J. (2009) Glutathione peroxidase-1 regulates mitochondrial function to modulate redox-dependent cellular responses. *J Biol Chem.* 284(18):11913-21.
- [44] Dominguez L, Sosa-Peinado A, Hansberg W. (2010) Catalase evolved to concentrate H₂O₂ at its active site. *Arch Biochem Biophys.* 500(1):82-91.
- [45] Ng CF, Schafer FQ, Buettner GR, Rodgers VG. (2007) The rate of cellular hydrogen peroxide removal shows dependency on GSH: mathematical insight into in vivo H₂O₂ and GPx concentrations. *Free Radic Res.* 41(11):1201-11.